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Current Perspectives on Nucleosome Positioning

Electrostatic Interactions between Arginines and the Minor Groove in the Nucleosome

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Abstract

Proteins rely on a variety of readout mechanisms to preferentially bind specific DNA sequences. The nucleosome offers a prominent example of a shape readout mechanism where arginines insert into narrow minor groove regions that face the histone core. Here we compare DNA shape and arginine recognition of three nucleosome core particle structures, expanding on our previous study by characterizing two additional structures, one with a different protein sequence and one with a different DNA sequence. The electrostatic potential in the minor groove is shown to be largely independent of the underlying sequence but is, however, dominated by groove geometry. Our results extend and generalize our previous observation that the interaction of arginines with narrow minor grooves plays an important role in stabilizing the deformed DNA in the nucleosome.

Introduction

The ability of proteins to bind preferentially to specific DNA sequences depends on a variety of readout mechanisms involving both the recognition of hydrogen-bonding and hydrophobic groups on different bases (base readout) and shape readout (1). The latter category includes sequence dependent deformability (2), intrinsic bending (3) and both global and local conformational variability (1, 4). We have recently identified an additional shape readout mechanism involving sequence dependent variations in minor groove width that is often associated with A-tract sequences (5). Readout is accomplished through the effect of minor groove shape on electrostatic potentials which are enhanced when the groove is narrow (6). Enhanced negative potentials are attractors for arginines which, when appropriately placed on the protein surface mediate specific protein-DNA interactions.

DNA binding to histones in the nuclesome core particle exploits this minor groove readout mechanism. Specifically, the periodic placement of stretches of between three and five As and Ts (often excluding the flexible TpA step) appears to play a role in bending the DNA around the histone octamer (7-9), producing narrow regions of the minor groove that face inward. There is enhanced electrostatic potential in these regions that are often found to bind arginines (6). Our previous examination of local shape readout in the nucleosome (6) was based in part on the analysis of a nucleosome structure formed by a human α-satellite DNA sequence and recombinant *Xenopus laevis* histones (PDB ID 1kx5) (10). Here we extend the analysis to two additional structures that vary in DNA or protein sequence. One structure contains histones from *Drosophila melanogaster* (PDB ID 2pyo) (11) with 16 amino acid substitutions with respect to the *Xenopus laevis* histones. The second structure contains multiple substitutions in its DNA

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relative to the reference human α-satellite DNA sequence, including a 16 base pair poly A:T sequence at base pairs 36-51 and the metal response element binding site for the transcription factor Amt1 at base pairs 66-74 (PDB ID 2fj7) (12). Comparing the three structures enables us to examine how differences both in the protein and DNA sequence affect arginine-DNA interactions in the nucleosome core particle.

We also consider the effect of salt on the electrostatic potential of DNA in the nucleosome. Numerical solutions to the nonlinear Poisson-Boltzmann (13) have been shown to provide an accurate description of the effect of salt on protein-DNA systems (14, 15) and, given the effect of salt on nucleosome dissociation (16) and the conformation of histone tails (17-19), it is of interest to see how the electrostatic potential of nucleosomal DNA is modified as salt concentration changes. Our results indicate that the recognition mechanism discussed here involving narrow minor grooves is largely independent of salt concentration, although the strength of histone-DNA interactions is strongly affected by salt.

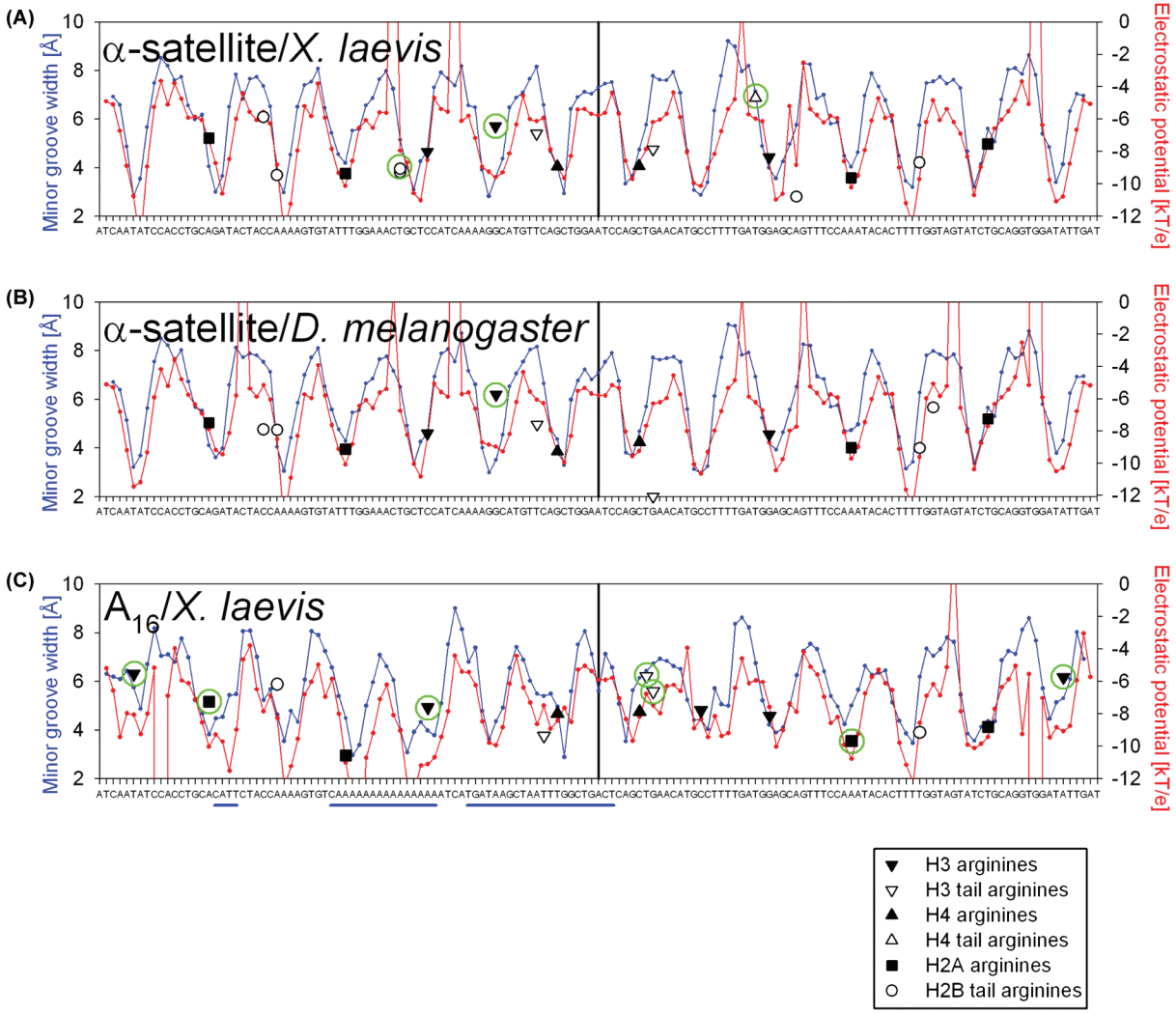

Figure 1: Minor-groove shape recognition in the nucleosome. Plot of the minor groove width and electrostatic potential for (**A**) DNA from the α-satellite/*X. laevis* histone structure, (**B**) DNA from the α-satellite/*D. melanogaster* histone structure, and (**C**) DNA from the A_{16}/*X. laevis* histone structure. Regions of the A_{16} DNA that vary in sequence from the α-satellite structures are underlined in blue. Arginines from each histone class are denoted with a marker as described in the legend. Arginine residues are placed at the base pair whose reference point is closest to their guanidinium Cζ atom. Arginines from the histone core are shown as filled markers; those in histone tails are shown as empty markers. Markers circled in green represent arginines located between 6.0 and 8.0 Å from the bottom of the minor groove. The vertical position of the markers is based on the most negative potential experienced by any atom of the guanidinum group.

Calculating Minor Groove Width and Helical Parameters

Minor groove geometry was analyzed for the 1kx5 (10), 2pyo (11), and 2fj7 (12) structures using the Curves 5.3 program (20). Values for minor grove width at each nucleotide were calculated by averaging the width at all given Curves levels. Helical parameters are shown as local parameters defined by Curves 5.3 (20). In comparison, structural parameters for canonical B-DNA were calculated for model structures produced by 3DNA based on fiber diffraction data (21). Accessibility of the grooves was visualized using GRASP2 surface representations (22).

Calculating Electrostatic Potential

Electrostatic potentials were obtained by solving the nonlinear Poisson-Boltzman equation using DelPhi (23-25). Unless otherwise noted, all calculations were done at physiological salt concentration of 0.145 M. Partial charges for the DNA and atomic radii for the protein and DNA were taken from the AMBER force field (26). The interior of the solute molecule, determined by using a probe sphere radius of 1.4 Å to find the solvent accessible surface, was assigned a dielectric constant of $\varepsilon = 2$, while the aqueous solvent was assigned a dielectric constant of $\varepsilon = 80$. Five focusing steps were used with Debye–Hückel boundary conditions for the initial step while focusing boundary conditions were used for the subsequent 4 steps (5).

The electrostatic potential in the minor groove is plotted as a function of DNA sequence based on the measurement of potential at reference points that are associated with base pairs. The reference point *i* is located midway along a line that connects the O4' sugar atoms in nucleotide *i*+1 in chain I and nucleotide *i*-1 in chain II. This geometric midpoint *i* lies approximately in the plane of base pair *i* and is approximately located in the center of the minor groove at about a distance of 3 Å from the edges of the base pair. Where the DNA strongly bends into the major groove the reference point can clash with the base functional groups and cause large shifts in electrostatic potential values.

Results

Figure 1 plots minor groove width and electrostatic potential as a function of base sequence for the three nucleosome core particle structures. The potentials are calculated at a reference point in the center of the minor groove with one reference point associated with each base pair (see Methods). The placement of arginines along the nucleotide sequence is here defined by the closest distance between the reference point and the Cζ atom of the arginine. Although not clearly defined, the same reference point was used to measure arginine positions in the minor groove in our previous analyses (6) (other methods of determining arginine positions have been discussed (27)). Another addition to previously reported data (6) is the inclusion of the electrostatic potential measured at histone arginine positions, defined as the most negative potential experienced by any atom in the guanidinium group, usually a hydrogen atom of one of the NH2 groups.

As can be seen in the figure, the difference in minor groove width in all structures varies by about 6.0 Å from the narrowest regions to the widest, while the electrostatic potential varies by about 8.0 kT/e. The shape of the DNA is very similar in both α-satellite structures (Figures 1a and 1b), and the electrostatic potential mirrors these similarities. There are,

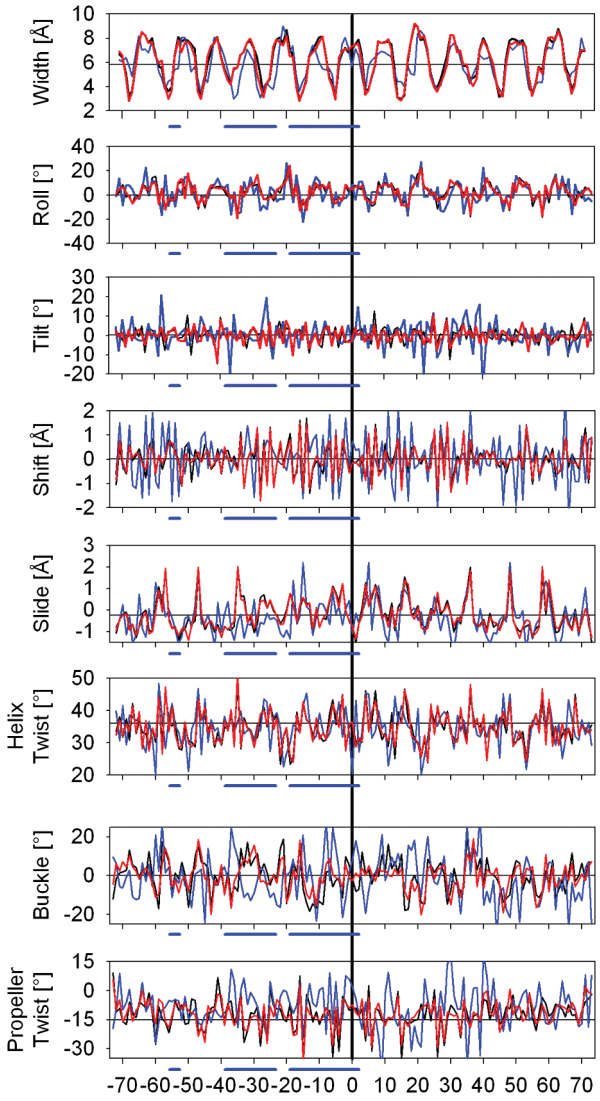

Figure 2: Minor groove width and helical parameters of the α-satellite/*X. laevis,* α-satellite/*D. Melanogaster* and A_{16}/*X.laevis* DNA structures. DNA structural parameters are plotted in red for the α-satellite/*X. laevis* structure, in black for the α-satellite/*D. melanogaster* structure and in blue for the A_{16}/*X. laevis* structure. Local parameters as defined by Curves are shown for helical parameters. Regions where the sequences vary between α-satellite and A_{16} are underlined in blue. Horizontal black lines indicate helical parameters for canonical B-DNA modeled from fiber diffraction data.

however, differences in sequence and shape between the α-satellite structures and A_{16} structure (Figure 1c) which affect the electrostatic potential. This is most notable in the long A-tract region, where the minor groove is narrower in the A_{16} structure and exhibits enhanced negative electrostatic potential.

As we found previously, arginines tend to be located in regions where the minor groove is narrow (6). In order to include arginines that are near the groove but do not penetrate into it, we use an 8.0 Å cutoff to the bases as a criterion for including an arginine in the figure (previously we used a 6.0 Å cutoff). As expected, most arginines located 6.0-8.0 Å from base atoms on the bottom of the groove (circled in green in Figure 1) experience a weaker potential than arginines intruding the groove.

Each histone that has arginines inserted into the minor groove, and whether the arginine is located on the histone core or tail is indicated in Figure 1. Arginines are inserted into the majority of the 14 narrow minor groove regions in each of the three structures; based on our original criterion for groove intrusion (6.0 Å from the bases), both α-satellite structures have arginines in 10 of their narrow regions and the A_{16}/*X. laevis* structure has arginines in 7 of its narrow regions. Expanding the distance criterion to 8.0 Å increases these numbers to 11 and 13 arginine-contacted narrow minor groove regions, respectively.

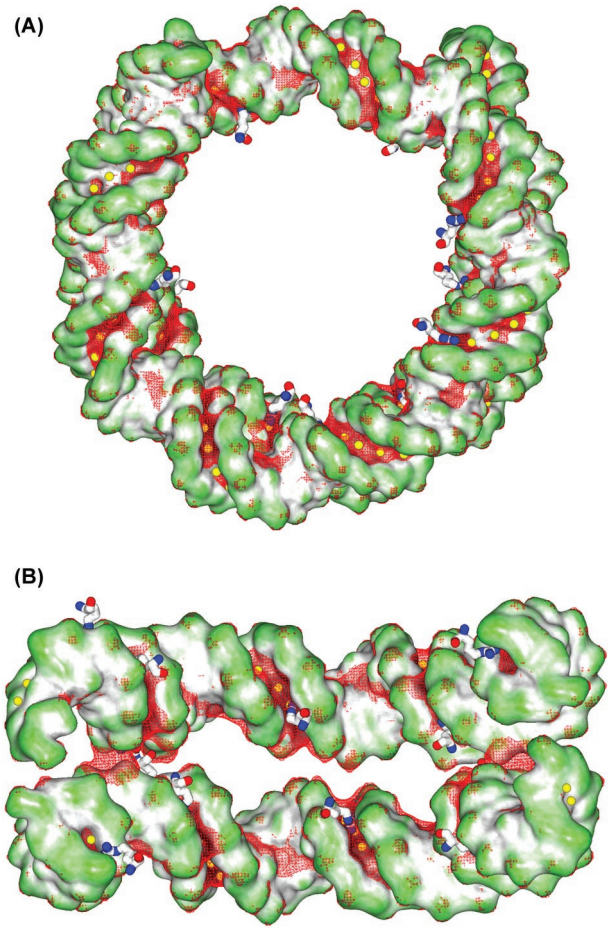

Figure 3: Electrostatic isosurfaces for nucleosomal DNA. GRASP2 surface representations (22) of the DNA taken from the α-satellite/*X. laevis* structures with (**A**) a view along the superhelical axis and (**B**) a view of a portion of the DNA facing the histone core are colored-coded for shape, with convex surfaces in green and concave surfaces in dark gray. Arginines are displayed as sticks and are located in the groove where the DNA is compressed. The yellow spheres represent the reference points inside of the minor groove where the electrostatic potential was measured. Mesh representations of the -6.0 kT/e isosurface are displayed in red. All arginines that contact the minor groove within a distance of 8.0 Å from the bases are shown.

To explore the factors that give rise to the minor groove widths seen in Figure 1, we calculated values of the geometric parameters that determine DNA shape. Figure 2 shows a comparison of minor groove width and helical parameters for each of the three structures. Analysis of helical parameters has previously been reported for the α-satellite structure (28). There is a clear periodicity of minor groove width in all three structures which is not seen when individual helical parameters are considered (except, to some extent, for roll. To a lesser extent, there is a correlation with slide and helix twist, especially at kinked base steps (2)). This highlights the importance of focusing on minor groove shape, which plays a clear functional role, rather than on individual helical parameters that can apparently combine in different ways to produce the same shape.

Figure 3 shows the *X. laevis* α-satellite structure in a view along the superhelical axis (a) and a view of a portion of the DNA facing the histone core (b) in GRASP2 (22) surface representation with the color of the surface determined by shape. The mesh representation of the isosurface of electrostatic potential, calculated for the DNA structure at -6.0 kT/e, shows how the enhanced negative electrostatic potential is recognized by arginines. The negative electrostatic potential is enhanced in minor groove regions where the DNA is compressed. Generally, the electrostatic potential becomes more negative towards the bottom of the minor groove.

Figure 4 plots electrostatic potentials along the nucleotide sequence for different salt concentrations. Although the absolute values of the potentials in the minor groove of the *X. laevis* α-satellite nucleosomal DNA are affected by the ionic strength, the difference in average values between the peaks and troughs only change by about 0.8 kT/e going from 0.001 M to 1.0 M salt concentration. Thus, effects of shape on electrostatic potential illustrated here are not affected by ionic strength. The structural details of the nucleosome core particle may, however, vary in different salt concentrations, which might in turn affect electrostatic potential. On the other hand,

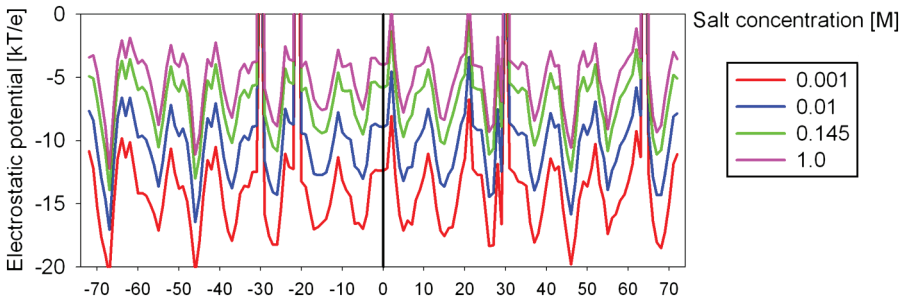

Figure 4: Effect of salt concentration on electrostatic potentials. Electrostatic potentials in the minor groove of the DNA from the α-satellite/*X. laevis* structure were obtained from solutions to the nonlinear Poisson-Boltzmann equation at varying salt concentrations. Electrostatic potentials are plotted at the center of the minor groove as a function of the base sequence.

the large effects on the magnitude of the electrostatic potential that are observed provide a basis for understanding the effect of salt on nucleosome stability.

Discussion

In this paper we have shown that there are biophysical design principles that are common to three different nucleosome structures. Specifically, all have narrow minor groove regions facing the histone core and, in each case, the negative electrostatic potential is enhanced in these regions. The effect is independent of base sequence in the sense that the potentials are due to DNA shape rather than to the functional groups that are presented by the bases in the groove. The enhancement of potential is also independent of changes in salt concentration. In each case arginines are often found in regions of enhanced negative electrostatic potential, providing a stabilizing interaction in the overall free energy balance of nucleosome formation.

Defining the location of arginines in the groove is fraught with ambiguities. The arginine side chain is quite long and different atoms may be close to different nucleotides. Since our focus is on electrostatic interactions, it seems reasonable to define the location of the arginine as corresponding to the center of the positively charged guanidinium group, *i.e.,* the Cζ atom (see also above). This helps identify the arginine location in terms of DNA shape. However, we do not take the assignment of an arginine to a base pair too literally, because the shape of the DNA is characteristic of a region and not of a particular base pair.

The importance of considering shape, in addition to the properties of individual nucleotides, is also apparent from consideration of the geometric variables plotted in Figure 2. Specifically, different sequences can yield similar groove widths despite quite different helical deformations. It is minor groove width that underlies electrostatic interactions with arginines rather than base-specific interactions as evidenced by the similar electrostatic profiles produced by different DNA sequences. There is still much to be learned about the energetics of nucleosome formation. Treating DNA as a molecule with a three-dimensional shape rather than as a one-dimensional string of letters may be an important component of future developments.

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